



Arch of Colors in Anatomic Pathology-Are They Most Significant in Inking of Surgical Margins?

Sonti Sulochana¹, Princy Mounika², Jalavadi Rahul Siddartha³,
Muthusubramanian⁴, N.V Subash Kolappan⁵

¹Professor, Department of Pathology, Saveetha Medical College and Hospital Thandalam, Chennai - 602105, India; ²MSc MLT, Tutor, Saveetha Medical College and Hospital, Thandalam, Chennai, Tamilnadu, 602105 India; ³MBBS, Stanley Medical College, Chennai, Tamilnadu, 602105 India; ⁴MD, Pathology, Tutor, Department of Pathology, Saveetha Medical College and Hospital, Thandalam, Chennai, Tamilnadu, 602105 India; ⁵Lab Manager, Histopathology, Department of Pathology, Saveetha Medical College and Hospital, Thandalam, Chennai, Tamilnadu, 602105 India.

ABSTRACT

Introduction: In surgical pathology various colours are used as a surgical ink margins. The pathologists are known of the various advantages that colored inks contribute to anatomic pathology practice. The margins of each excised gross specimen colouring with different colours, add correctness to margin examination. It showed a three-dimensional microscopic restoration of features of the tumor and also anatomic structures. The use of multiple colours are orientation of grossed and dissected specimens, to re-examine the grossed specimen by the pathologist, if required, and if necessary further sampling of the specimen and also allow the postoperative comparison of tissue planes anticipated by preoperative imaging.

Aim and Objective: A comparative study between Mashi(Indian Ink) and acrylic colors used as a surgical ink margin

Materials and Methods: The study was done on 350 surgically excised medium, large and formalin-fix specimens. The surgical margins of each specimen were marked with Indian ink and five acrylic colors. The grossed specimens were then processed using routine histopathology techniques and sections were stained with hematoxylin and eosin stain(H&E). The macroscopic and microscopic parameters were used to study the suitability of acrylic colors and Indian Ink (mashi) as surgical ink for margins.

Results: The study noticed specific shades of acrylic colors that are easily available and simple to use, with good results on macroscopic and microscopic examination.

Conclusion: Colored inks impart accuracy to margin examination. Acrylic colors are more suitable as surgical ink over Indian.

Key Words: Surgical pathology, Margins, Inks, Color, Specimens, H&E stain, Tumour

INTRODUCTION

In surgical pathology various colours are used as a surgical ink margins. The pathologists are known of the various advantages that colored inks contribute to anatomic pathology practice. Histopathological assessment of either wide or radical excision of surgical specimens requires proper orientation of the specimen along with the identification of all surgical margins.¹⁻⁸ Various methods have been utilized for margin assessment like the marking of surgical margins by applying Indian ink or with sutures and clips.^{1,2} In the area of surgical pathology, the Indian ink has long been used.¹⁻⁶ However, for identification and assessment of various margins, there has been a lack of use of multiple colored inks.^{1,2} The use of multiple color ink is useful in the following ways: 1) For the orientation

of a specimen, 2) For margin status 3) For the benefit of post-grossing three-dimensional restoration 4) To reduce identification error when multiple sampling is required from the same tissue or when obtaining similar specimens from different patients biopsies.^{1,2,6,7} Infact, during the HPE reporting, the vital decision of whether the margin is positive for tumor cells depends entirely on the presence or absence of ink⁸.

Although tissue marking dyes (TMD) are available on the market, they are expensive, more drying time and interference with IHC.^{2,4,9,10} Acrylic colors are easily available in numerous colors, and are cost-effective, non-flammable, non-toxic, and stable(Figure-1).^{1,4,6,9,11} However, not all acrylic colors withstand tissue processing and fulfil the criteria of being used as surgical ink.

Corresponding Author:

Sonti Sulochana, Professor, Department of Pathology, Saveetha Medical College and Hospital, Thandalam, Chennai - 602105, India.
Mobile: 08838877983; Email:sulochanamaster@gmail.com

ISSN: 2231-2196 (Print)

ISSN: 0975-5241 (Online)

Received: 16.03.2023

Revised: 09.05.2023

Accepted: 21.06.2023

Published: 23.06.2023

The objective of this study was to analyse commercially available acrylic colors and Indian ink for inking surgical margins.

MATERIALS AND METHODS

The study was done on 350 surgically excised and formalin-fixed specimens of small and large biopsies. Surgical margins of specimens were marked with Indian ink (HIMEDIA) and with acrylic colors. On the whole, five different contrasting acrylic colors were used to mark the different margins (Black, Red, Violet, Light Green, and Sky Blue).

The surface of the fixed specimens was dried with blotting or tissue paper. The painting of the specimens with acrylic colors and Indian Ink was done using a commercially available brush (Cameclin limited white bristle brush, size: 4). The painted specimens were immersed in alcohol for a few minutes or aired to fix the color on the surface. The excess color was washed off and then the specimen was grossed out as done routinely. The surgical margins were grossed out from each small and large specimen and then processed using routine histopathology techniques, i.e. formalin-fixed - paraffin embedding techniques. Each specimen was processed separately mainly to examine the contamination of processing fluids. The thickness of the tissue section of 4 μ m was cut from each block and stained with haematoxylin and eosin stain (H&E).

The dyes/colored inks were assessed on the following parameters:

1. Inking of margins with various colours-is it ease or difficult application?
2. Drying time of ink-is it short or long?
3. The other tissues and processing fluids-are they contaminated or not?
4. Visibility of colour on paraffin blocks, on the slide, and under the microscopy-is it clear or hazy?
5. Seepage into deeper tissue and interference with nuclear and cytoplasmic morphology.

The study was approved by institutional ethics committee (SMC/IEC/2020/02/004).

RESULTS

The results were interpreted by all the above parameters and are shown in Table 1

In anatomic pathology, inking tissue margin was a method for proper identification of surgical margins along with margin status holds the greatest importance. Mashī has long been used but it can ink only by one margin (Fig-1). On the other hand, acrylic colors, available in different colours (Fig-2) can be used for inking multiple margins along with many more advantages. The present study was undertaken to analyse acrylic colors and Mashī for inking surgical margins.

The specimens were painted with acrylic colors (350) and Indian ink (350) using a commercially available brush by two different technicians. Both of them noticed equal ease of application for both the samples. (Fig3)

The time required for the drying of the acrylic dye and India ink (2.00 ± 0.05 minutes) and drying period for the different colors of acrylic dye was equal. The blocks made for acrylic colors and Indian ink showed clear visibility on paraffin blocks (Fig4).

The macroscopic and microscopic examination of surgical margins was clearly visible for both India ink and acrylic colours (Fig 5, 6). But no interrupted staining on microscopic examination. Most importantly, all acrylic colors (100%) and Indian ink were visible very clearly on macroscopic and microscopic examination. Our study correlated with Tampi and Chiam.^{1,4}

Indian ink and acrylic colours have no contamination or staining of other tissues, processing fluids and solutions were reported. A detailed microscopic examination of the interphase between the dye and surgical margin revealed very minimal seepage of acrylic and Indian ink dye into the tissue and was only observed at the margins of the tissue section, leaving all other areas unaffected. Hence, there was no interference with the microscopic interpretation of nuclear and cellular details.

In acrylic colors, the parrot green color and sky blue color are looking slightly similar under microscopic examination. So instead of sky blue, better use dark blue color or avoid use of any one colours. The red color in some areas penetrated in to the blood vessels as a hemorrhagic material.

DISCUSSION

Nowadays the inking of the surgical margins of the malignant tumours is very important for staging, grading and prognosis of the disease. For notifying the surgical margins before and after tissue processing, the use of multiple colors or pigments more reliable than sectioning techniques or suture markers¹ Microscopic evaluation of the margins of excised tumor specimens is of more important for identification of the infiltration of tumor cells or tissue do not cross the edge of the margin.

Indian ink, a colloidal suspension of inert carbon black, has been in use in India since the fourth century BC, where it was called "Mashī" (Tamil).¹ Jain sutras and several documents that were written with this ink have been unearthed intact as far as China. The carbon black was obtained by burning tar pitch, bones, etc., and then mixed with resin/gum/shellac for binding and with water-ethylene glycol as a medium for the emulsion.

Most of the required qualities are present in India ink for inking surgical margin. It sustained in tissue processing and staining procedures and finally when viewed under the microscope, the black ink line is seen following the edges of the tissue. Acrylic paints were first used in the 20th century in Mexico. Acrylics are the most popular paint today because they are cost-effective, easy to dilute with water, they dry quickly and they are resistant to aging and smudging. Acrylic paints are generally pigments suspended in acrylic polymer emulsion, that is, color pigments in a mixture of multiple chemicals as a thick emulsion.^{1, 6,9,11}

Acrylic color pigments are not translucent, so we can paint in layers and won't see the previous color through it. The acrylic paint brushes, hands, and other equipment clean with water and soap for manifold to utilize.¹² In the present study, we assessed acrylic paint and Indian ink could be ease, rapidly to applied, and readily identified in the histologic section by the pathologists.

In the present study, we used the black, red, blue, green, and purple colours, which fulfil the criteria for ideal surgical margin ink. It was observed that these acrylic colors fix fresh and formalin-fixed tissue easily. They undergo overnight processing and staining procedures and can be seen on paraffin blocks as well as on sections. Similar results were reported by Tampa C¹ and Chiam HW.⁴ Indian ink also showed similar results. The Drying time for acrylic colors and Indian ink was 2.00 ± 0.05 . But other studies showed that the time required for the drying of the acrylic dye (1.30 ± 0.009 minutes) was significantly less than Indian ink (2.30 ± 0.05 minutes). Hence, Sachin C Sarode² study was not correlated with our study.

Acrylic colors and Indian ink do not unduly color the processing fluids or spread within the tissue sections. The big advantage of acrylic color is its availability in most stationery shops, it can be bought in single units of variable colors, have a long shelf life, is more cost-effective, and is nontoxic.^{1,2} Indian ink is not available across the counter, is available in a single color, expensive and is toxic.

The acrylic pigments were easily visualized on paraffin blocks and microscopically at the surgical margins. Different colors distinctly stood out at all levels. These results are from the study conducted by Tampa C¹, 2012; and Chiam H W.⁴ Thus, they fulfil all the criteria of being a reliable, easy to use, inexpensive, safe, and stable substitute for Indian ink.

Nowadays, in our laboratory, these specific acrylic hobby colors are in regular use to mark the different surfaces in wide excision specimens of soft tissue tumors with remarkably good and dependable results. The greatest advantage of acrylic color over Indian ink is that we can choose different colors for different margins. This will help the pathologist in the re-examination of grossed specimens very effectively

and he can identify the surgical margins without any difficulty. The same is true for microscopic examination; the microscopic visibility of a particular color gives us the idea of the type of margin being examined. The most advantage of acrylic colour is do not interfere with immunohistochemical stains.^{1,6,11}

CONCLUSION

The Indian ink and Acrylic colors have similar features like ease of application, faster drying time, no penetration and contamination of processing fluids, other tissues, excellent clarity on paraffin blocks and under a microscopic study.

We finally conclude that the acrylic colors are more suitable as surgical margin ink over Indian ink because of its easy availability in different colors, inexpensive, and safe. These colours do not impede with special stains or immunohistochemistry (IHC) studies.

Hence, we propose acrylic colours use as a routine dye for marking surgical margins in various tissue specimens and also useful in inking of external surface with different colours and identification of different parts of internal organs in anatomy dissection.

ACKNOWLEDGEMENT

The authors are thankful to management, HOD, IEC committee for their support and advice. And also we express our gratitude to the teaching and non-teaching staff of department of pathology for their constant encouragement and guidance. We are thankful to all the authors, whose articles are cited and included in references of this manuscript.

Source of Funding: Self

Conflict of Interest: None

Authors' Contribution: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

REFERENCES

1. Tampi C. In search of the rainbow: Colored inks in surgical pathology. *Indian J Pathol Microbiol.* Apr-Jun 2012; 55(2):154-7
2. Sarode SC, Sarode GS, Patil S, Mahajan P, Anand R, Patil A. Comparative Study of Acrylic Color and India ink for their use as a surgical margin inks in oral squamous cell carcinoma. *World J Dent* 2015; 6 (1):26-30.
3. Shinde V, Phelan C, Gater W, Thomas J. Inking a specimen without the mess. *J Clin Pathol.* July 2008; 61(6):783.
4. Chiam HW, Maslen PG, Hoffman GJ. Marking the surgical margins of specimens: Commercial acrylic pigments are reliable, rapid and safe. *Pathology.* 2003 Jun; 35(3):204-6.

5. Paterson DA, Davies JD. Marking planes of surgical excision on breast biopsy specimens: Use of artists' pigments suspended in acetone. *J Clin Pathol.* 1988 Sep; 41(9):1013-6.
6. Ranjan R, Singh L, Arava SK, Singh MK. Margins in skin excision biopsies: Principles and guidelines. *Indian J Dermatol.* 2014 Nov;59(6):567-70.
7. Parkinson AB, Cannon CR, Hayne ST. Colour coding surgical margins with the Davidson marking system. *J Histotechnol.* 1990; 13:293-294.
8. Westra WH, Hruban RH, Phelps TH, Isacson C. Surgical pathology dissection: An illustrated guide. Chapter 1, General approach to surgical pathology specimens; 2nd ed. New York: Springer; 2003. p. 2-13.
9. Williams AS, Dakin Haché K. Variable fidelity of tissue-marking dyes in surgical pathology. *Histopathology.* 20 Jan 2014, 64(6):896-900.
10. Wong JW, Bai H, Abdul-Karim FW, MacLennan GT. Simulation of micro calcifications on specimen radiographs of breast biopsies by inks used in marking the surgical resection margins. *Breast Journal.* Sep-Oct 2004;10(5):423-6.
11. Williams AS, Hache KD. Recognition and discrimination of tissue marking dye color by surgical pathologists recommendations to avoid errors in margin assessment. *Am J Clin Pathol.* 01 Sep 2014; 142(3):355-361.
12. Oscar C, Scalarone D, Learner T. Characterization of Artists' Acrylic Emulsion Paints. *Int J Polymer Anal Char* 2003; 8:67-82.

Table 1: Interpretation of Results

S.no	Parameters	Ink	Cases	Result
1	Ease of application	Indian ink	350	100%
		Acrylic	350	100%
2	Drying time of Ink	Indian ink	350	2.00±0.05mts
		Acrylic	350	2.00±0.05mts
3	Contamination of other tissues and processing fluids	Indian ink	350	None
		Acrylic	350	None
4	clearness on paraffin blocks	Indian ink	350	100%
		Acrylic	350	100%
5	clearness on naked eye examination of slides	Indian ink	350	100%
		Acrylic	350	100%
6	Clarity on microscopy	Indian ink	350	100%
		Acrylic	350	100%
7	Seepage into deeper tissue and interference with nuclear and cytoplasmic morphology	Indian ink	350	None
		Acrylic	350	None

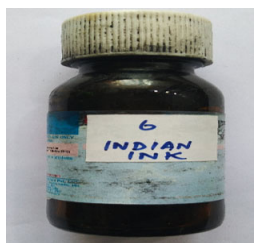


Figure 1: Indian ink.

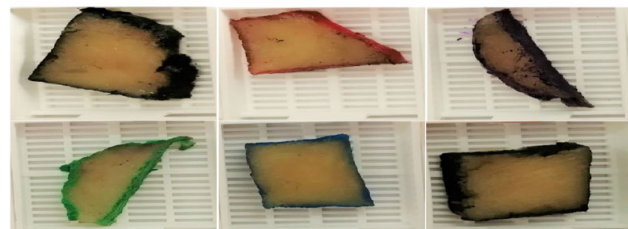


Figure 3: Clear visibility of Acrylic colours(1 to 5 black, red, purple, green, blue) and Indian Ink(6,) on tissue margins.



Figure 2: Acrylic Colours.

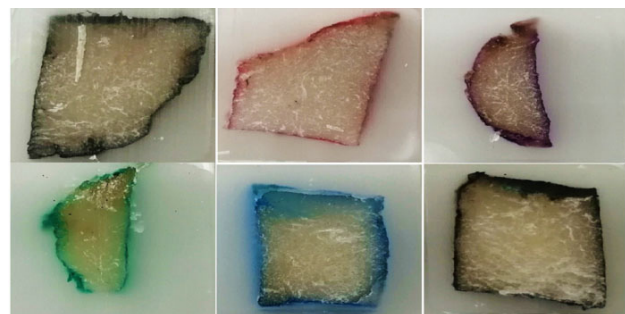


Figure 4: Excellent clarity of Acrylic colours and Indian Ink on paraffin block.

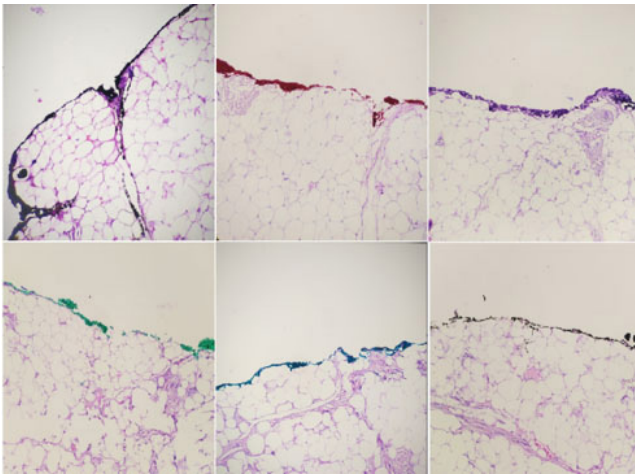


Figure 5: Excellent clearness of Indian Ink and Acrylic colours on Microscopic view.



Figure 6: Gross specimen of duodenum with pancreas. The pancreatic tumour coloured with green acrylic colour.